Original Article

Diagnostic Implications Of Urine 8-Hydroxy-2-Deoxyguanosine (8-Ohdg) As A Sensitive Biomarker For Early Prediction Of Diabetic Kidney Disease Among Adolescents With Type 1 Diabetes Mellitus

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Abstract

Background: The most prevalent and severe microvascular complication in individuals who have type 1 diabetes (T1DM) is diabetic kidney disease (DKD), and its severity is assessed by the degree of albuminuria, but it lacks the sensitivity and specificity required to identify DKD in an early stage; necessitating the use of other biomarkers. One of these biomarkers is 8-Hydroxy-2-Deoxyguanosine (8-OHdG) in urine. **Aim:** To evaluate urine 8-OHdG's sensitivity and specificity for earlier identification of DKD. **Methods:** Ninety-one study participants were recruited as 64 adolescents with T1DM and 27 healthy control subjects. According to the results of their urine albumin/creatinine ratio (UACR), T1DM patients were classified into three groups: normoalbuminuric group, intermittent microalbuminuric group, and persistent microalbuminuric group. Blood pressure and anthropometric measurements were performed. Laboratory tests were requested within 3 months of T1DM patient recruitment. All participants' urine samples were taken at the time of their enrollment, and ELISA kits were used to check for urine 8-OHdG. **Results:** Mean glycated hemoglobin (HbA1c) was above the target level (≥7%) in 96.9% of participants with T1DM. T1DM patients' mean urine 8-OHdG was considerably greater in comparison to the control group, additionally those with persistent or intermittent microalbuminuria also had higher mean levels than those with normoalbuminuria. There was highly statistically considerable difference between the three groups of T1DM regarding urine 8-OHdG. We found that urine 8-OHdG is unable to discriminate between persistent and intermittent microalbuminuria, but can fairly discriminate between normoalbuminuria and persistent microalbuminuria, as well as normoalbuminuria and intermittent microalbuminuria. **Conclusion:** Nine T1DM patients with normoalbuminuria had urine 8-OHdG levels higher than the cutoff limit, so longitudinal follow up of those patients is recommended to make sure that they will develop microalbuminuria later on, and at that time we can prove that urine 8-OHdG can be used for earlier detection of DKD.

Key words: Type 1 diabetes, Diabetic microvascular complications, Diabetic kidney disease, Albuminuria, Urine 8-OHdG biomarker.

Introduction

One of the most significant long-term diabetes microvascular complications is diabetic kidney disease (DKD) **(Uwaezuoke and Ayuk, 2020).** It is defined by the following features: persistent albuminuria identified in at least two out of three urine samples tested for urine albumin/creatinine ratio (UACR); taken during three to six months, hypertension, and progressive decline in glomerular filtration rate (GFR) **(Donaghue et al., 2018)**. UACR < 30 mg/g creatinine is considered normal, UACR between 30 and 300 mg/g creatinine is considered microalbuminuria, and UACR greater than 300 mg/g creatinine is considered macroalbuminuria **(ElSayed et al., 2023).**

Over a period of 10 to 20 years, DKD progresses from microalbuminuria to end-stage renal disease (ESRD) **(Zhang et al., 2018)**, in addition to high risk of cardiovascular morbidity **(MacIsaac et al., 2014)**. DKD occurs in up to 40% of people with T1DM. Since a large percentage of diabetic patients have reported spontaneous remission of their microalbuminuria, recent research has questioned the accuracy of microalbuminuria. New biomarkers are therefore required for the early identification of DKD and prevention of progression to ESRD **(García-Carro et al., 2021).**

Studies have demonstrated that oxidative stress caused by hyperglycemia in diabetic individuals results in reactive oxygen species (ROS) synthesis **(Giacco and Brownlee, 2010).** Normally, there is a harmony between the synthesis of oxygen-free radicals and the antioxidant defense mechanisms **(Elmarakby and Sullivan, 2012).** Clinically, plasma ROS biomarker levels are elevated in diabetic patients whereas antioxidant agent and enzyme levels are decreased **(Johar and Bernstein, 2017).** Urine 8-Hydroxy-2-Deoxyguanosine (8-OHdG) is tested to evaluate oxidative stress in the human body

SUBJECTS AND METHODS

Study participants

Ninety-one participants were included in this study; 64 adolescents with T1DM were enrolled from the pediatric endocrine unit at the Cairo University Children's Hospital, and 27 healthy adolescents served as the control group. Recruitment took place between November 2020 and March 2021 for both groups. According to the findings of the urine albumin/creatinine ratio (UACR), patients with T1DM were classified into three groups: 29 normoalbuminuric patients (UACR <30 mg/g creatinine), 15 intermittent microalbuminuric patients (UACR ≥30-300 mg/g creatinine in intermittent manner), and 20 persistent microalbuminuric patients (UACR ≥30-300 mg/g creatinine in minimum two out of three urine samples tested for UACR; taken within three- to six-month period). At the time of recruitment, the study participants' ages ranged from 10.2 to 19 years, and their mean ages were 14.84 ± 2.59 years. The study included those with T1DM for at least five years, and both genders. Patients who have type 2 diabetes or monogenic diabetes, diabetes duration less than 5 years, age less than 10 years or more than 19 years, patients with any other diseases (if unrelated to diabetes) such as: cardiovascular, renal, urinary tract, hepatic, respiratory, neurodegenerative, psychiatric diseases, and overweight or obesity were excluded.

Ethical approval

The Ethical Committee of Cairo University's Faculty of Medicine gave its approval to the study. The approval code from the Ethical Committee is MD-91-2020.The study objectives, steps, and potential benefits were discussed with caregivers of all study participants or their children if older than 12 years. All participants were enrolled after receiving their caregivers' informed consent.

Methods:

The control group was subjected to history taking, anthropometric measurements, and laboratory testing. History taking included age, gender, and history of any illnesses or drug use. Anthropometric measurements including weight

and height were assessed and interpreted using WHO international growth charts. Blood pressure (BP) measurement was performed using a mercury sphygmomanometer in the sitting position with a cuff of appropriate size during the patients' visit and after 10 minutes of rest and the measurements were plotted on the Houston Pediatric and Adolescent Hypertension Program (HPAHP) BP charts **(Banker et al., 2016)**. Using ELISA kits, urine samples were obtained and analysed for urine 8-Hydroxy-2-Deoxyguanosine (8-OHdG).

T1DM patients who met the inclusion criteria, were also subjected to history taking, physical examination, and laboratory evaluation. History taking included age, gender, age at onset of T1DM, duration of T1DM, history suggestive of other diabetic complications (retinopathy and neuropathy), and history of other diseases (cardiovascular, renal, urinary tract, hepatic, respiratory, neurodegenerative, and psychiatric diseases). Physical examination included anthropometric measurements (as the control group), BP measurement, and full system examination to exclude other complications of T1DM and other chronic diseases.

The following laboratory tests were obtained from patients' files with last ones were performed within 3 months of patient recruitment, except urine 8-OHdG test that was done at the time of patient recruitment. Glycated hemoglobin (**HbA1c)** target is <7% (the most recent one was within 3 months from the current study) **(DiMeglio et al., 2018).** In order to evaluate glycemic control, HbA1c values from the five years prior to the study were also checked from the patients' files.

Urine culture was also done before measurement of UACR and urine 8-OHdG level to exclude urinary tract infections to avoid false positive results for albuminuria and in order not to affect the results of urine 8-OHdG.

Because albumin excretion varies throughout the day, first-voided urine in the morning was tested for UACR **(Donaghue et al., 2018)**. Independent of kidney damage, UACR may be increased by infection, marked hyperglycemia, menstruation, exercise, fever, and marked hypertension **(Tankeu et al., 2017).**

Schwartz formula was used to calculate eGFR (eGFR k (height in cm) ÷ serum creatinine that was measured within 3 months of the current study); k = 0.55 in children up to the age of 13 years and 0.7 in adolescent males (females continue to have a k of 0.55 after the age of 13) **(Schwartz and Work, 2009).** Both genders aged 10 to <13 were assessed to have normal eGFR of 133 ± 27 ml/min/1.73 m^2, males aged 13 to 19 were considered to have normal eGFR of 140 ± 30 ml/min/1.73 m^2, and females aged 13 to 19 were considered to have normal eGFR of 126 ± 22 ml/min/1.73 m^2 **(NKF KDOQI, 2002).**

Urine 8-OHdG was tested using 8-OHdG ELISA kits. Urine samples were collected and centrifuged. After being separated, the supernatant was kept at - 80°C. Urine 8-OHdG levels were measured using ELISA kits. The used device is **Stat Fax-2100.**

Statistical analysis:

Numerical data were summarized using mean, standard deviation, median and range. Categorical data were summarized as percentages. The normal assumption was tested by the Kolmogorov Smirnov. Comparison between the two groups with respect to normally distributed numeric variables were done by the independent t-test. Non normally distributed numeric variables were compared by Mann-Whitney test. For normally distributed data, ANOVA test was used to compare the normoalbuminuric, persistent microalbuminuric, and intermittent microalbuminuric groups. When data deviates from the normal assumption, comparisons were made using the Kruskal Wallis test and the Mann Whitney test as post hoc multiple 2-group comparisons. To compare categorical data, a Chi-square (χ^2) test analysis was performed. Exact test was used when anticipated frequency was <5. Correlations between numeric variables were determined by Pearson's test. Sensitivity and specificity were used to describe the accuracy. To determine the cutoff value for the investigated marker, receiver operator characteristic (ROC) analysis was used. P

values less than 0.05 were considered significant. Statistical analysis was carried out using the Statistical Package for the Social Science (SPSS) version 22.

RESULTS

At the time of recruitment, the ninety-one study participants' ages ranged from 10.2 to 19 years, and their mean ages were 14.84 ± 2.59 years. From a total of 64 patients with T1DM, males constitute 40.6% (n=26) and females constitute 59.4% (n=38). 9.4% of T1DM patients were underweight (weight SDS <-2) and 25% of them were stunted (height SDS <-2).

		Normoalbuminuric group n=29(%)	Intermittent microalbuminuric group n=15(%)	Persistent microalbuminuric group n=20(%)	p value between normoalbuminuric and intermittent microalbuminuric groups	p value between normoalbuminuric and persistent microalbuminuric groups	p value between intermittent and persistent microalbuminuric groups
Age:	**Mean ± SD** **Median** **Range**	14.95 ± 2.24 14.2 11.5-18.9	16.46 ± 2.34 17.3 12.4-18.8	15.15 ± 2.38 14.6 10.2-18.7	0.113	0.569	**0.133**
Gender:	**Male** **Female**	11 (37.9%) 18 (62.1%)	7 (46.7%) 8 (53.3%)	8 (40%) 12 (60%)	0.576	0.884	**0.693**
Weight SDS:	**Mean ± SD** **Median** **Range**	-0.007 ± 0.92 -0.1 -1.89-1.53	-0.03 ± 1.08 0.38 -1.9-1.53	-1.13 ± 1.36 -1.27 -3.17-0.99	0.833	**0.006**	**0.120**
Height SDS:	**Mean ± SD** **Median** **Range**	-0.55 ± 1.09 -0.36 -3.67-1.24	-0.98 ± 1.11 -1.32 -3.08-1.06	-1.95 ± 1.49 -1.73 -4.42-0.35	0.134	**0.003**	**0.072**

The difference between normoalbuminuric and persistent microalbuminuric groups in terms of weight and height SDS was statistically significant (p = 0.006 and 0.003, respectively) (**table 1**).

Table (1) Clinical and anthropometric data in patients with T1DM

Mean age at T1DM onset was 5.46 ± 2.51 years, and mean duration of TIDM was 9.9± 2.71 years. The three T1DM groups did not differ in these variables in a statistically meaningful way (**table 2**)

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Table (2) Age at onset and duration of T1DM

	Mean ± SD	Median	Range
Age at onset of T1DM (years)	5.46 ± 2.51	5.2	**0.9-10.5**
Duration of T1DM (years)	9.9 ± 2.71	9.95	**5.1-17**

In 62 of the T1DM patients (96.9%), the HbA1c was higher than the target level (≥7%). Mean HbA1c in the past five years was above the target level (≥7%) in 96.6% of normoalbuminuric group, 93.3% of intermittent microalbuminuric group, and 100% of persistent microalbuminuric group (**table 3**). There was no statistically significant difference between the 3 groups regarding mean HbA1c. The highest mean HbA1c in the previous five years was 16.2%. There was weak positive correlation between urine albumin/creatinine ratio and mean HbA1c in the past five years (p = 0.049 and r = 0.247)**.**

Glycemic control	Normoalbuminuric group n=29(%)	Intermittent microalbuminuric group n=15(%)	Persistent microalbuminuric group n=20(%)
Within the target (mean HbA1c <7%)	1 (3.4%)	1 (6.7%)	**0 (0%)**
Above the target (mean HbA1c ≥7%)	28 (96.6%)	14 (93.3%)	**20 (100%)**

In T1DM patients, the urine 8-OHdG range was 10.9 to 49.3 ng/ml and the median was 23 ng/ml, while in the control group, its range was 7.5 to 15.7 ng/ml and the median was 10.4 ng/ml **(table 4)**. Regarding urine 8-OHdG, there was a statistically considerable difference between patients with T1DM and the control group, and also between the 3 groups of T1DM patients (p<**0.001**); as demonstrated in **table 5**. The differences between normoalbuminuric and intermittent microalbuminuric groups (p=**0.045)** and normoalbuminuric and persistent microalbuminuric groups (p=**0.021**) were statistically significant**.** Additionally, there was intermittent versus persistent microalbuminuric groups difference that was highly statistically significant (p<**0.001**).

Table (4) Urine 8-OHdG in T1DM patients and the control group

	T1DM patients	Control
Urine 8-OHdG (ng/ml):		
Mean ± SD	24.71 ± 9.62	10.98 ± 1.92
Median	23	10.4
Range	10.9-49.3	7.5-15.7

Table (5) Comparison between the three T1DM groups and the control group regarding urine 8-OHdG

	Normoalbuminuric group	Intermittent microalbuminuric group	Persistent microalbuminuric group	Control group	p value (T1DM patients and control)	p value (3 groups of T1DM)
Urine 8-OHdG (ng/ml):						
Mean ± SD	20.27 ± 5.66	26.03 ± 7.48	30.15 ± 12.49	10.98 ± 1.92	**< 0.001**	**< 0.001**
Median	19.4	24.7	25.95	10.4		
Range	10.9-33.8	15.6-43.1	14.7-49.3	7.5-15.7		

In T1DM patients, we found a strong positive correlation between urine 8-OHdG and urine albumin/creatinine ratio ($p<0.001$ and $r=0.530$) **(figure 1).**

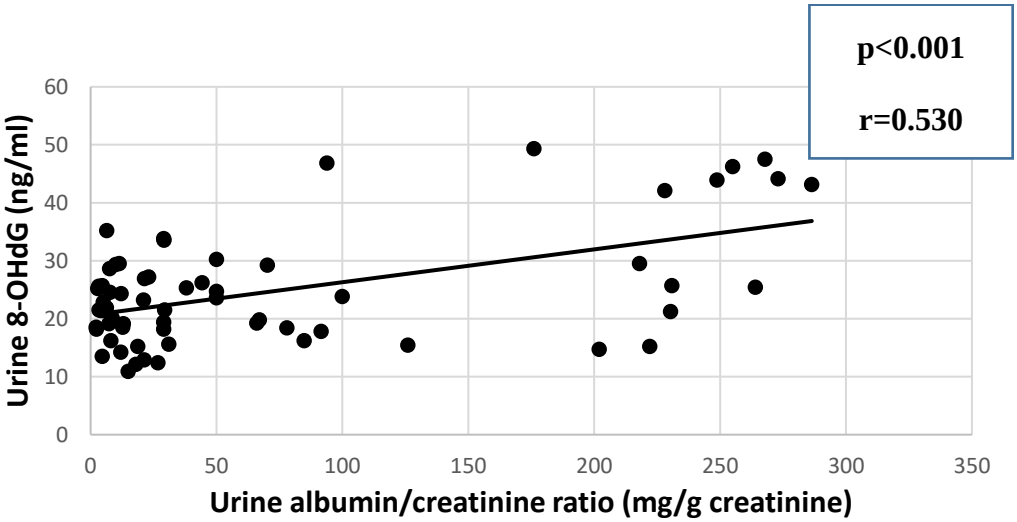

Figure (1) Correlation between urine 8-OHdG (ng/ml) and urine albumin/creatinine ratio (mg/g creatinine) in T1DM patients

Table (6) Correlation between urine 8-OHdG and mean HbA1c in the last 5 years and eGFR
Urine 8-OHdG had no statistically significant correlation to any of these variables.

	Urine 8-OHdG (ng/ml)	
	p value	**r value**
Mean HbA1c in the last 5 years (%)	0.056	0.661
eGFR (ml/min/1.73 m^2)	0.888	-0.018

We performed ROC analysis to determine the best cutoff value for urine 8-OHdG to differentiate between the 3 groups of T1DM. We found that urine 8-OHdG can fairly distinguish between normoalbuminuric and intermittent microalbuminuric groups (area under the ROC curve = 0.726 = 72.6%). The best cutoff limit was 23 ng/ml, which had a 66.7% sensitivity and a 65.5% specificity (**figure 2**). We also found that urine 8-OHdG can fairly distinguish between normoalbuminuric and persistent microalbuminuric groups (area under the ROC curve = 0.729 = 72.9%). The best cutoff limit was 23.3 ng/ml, which had a 65% sensitivity and a 69% specificity; as demonstrated in **figure 3**, but we found that urine 8-OHdG can't distinguish between intermittent and persistent microalbuminuric groups (area under the ROC curve = 0.565 = 56.5%) (**figure 4**).

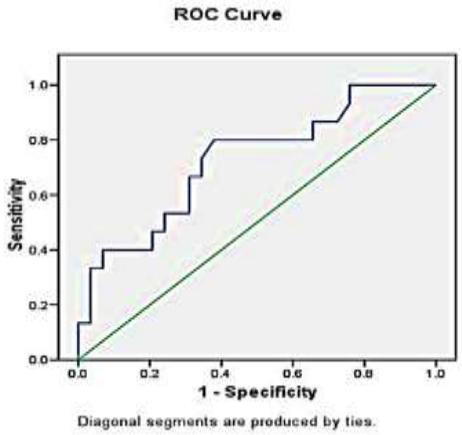

Figure (2) ROC analysis for discriminating normoalbuminuric and intermittent microalbuminuric groups regarding urine 8-OHdG

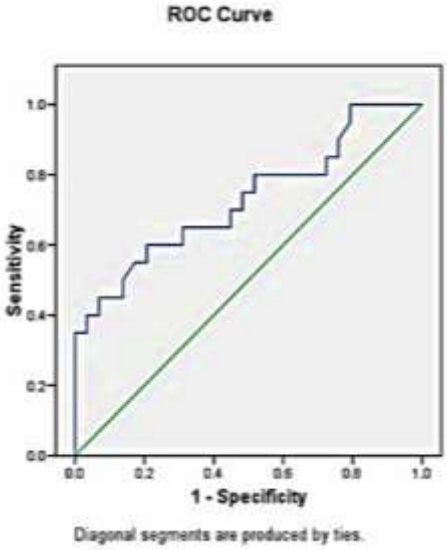

Figure (3)ROC analysis for discriminating normoalbuminuric and persistent microalbuminuric groups regarding urine 8-OHdG

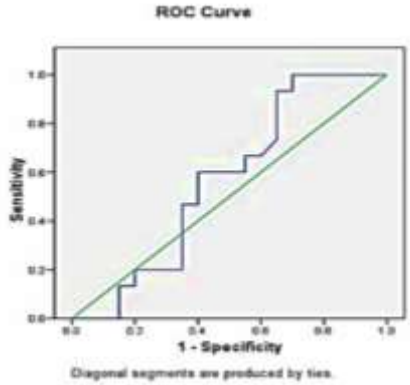

Figure (4) ROC analysis for discriminating intermittent and persistent microalbuminuric groups regarding urine 8-OHdG

There was no statistically significant difference in blood pressure among the three T1DM groups (**table 7**).

Table (7) Blood pressure (SBP and DBP) centiles in the three T1DM groups

	Normoalbuminuric group n=29(%)	**Intermittent microalbuminuric group n=15(%)**	**Persistent microalbuminuric group n=20(%)**	**p value**
SBP (centiles):				
♦ < 5th	2 (6.9%)	2 (13.3%)	1 (5%)	
♦ ≥5th - <90th	22 (75.9%)	11 (73.3%)	17 (85%)	0.957
♦ ≥90th - <95th	2 (6.9%)	1 (6.7%)	1 (5%)	
♦ ≥95th - 99th	3 (10.3%)	1 (6.7%)	1 (5%)	
DBP (centiles):				
♦ < 5th	0 (0%)	1 (6.7%)	0 (0%)	
♦ ≥5th - <90th	27 (93.1%)	13 (86.7%)	17 (85%)	0.452
♦ ≥90th - <95th	0 (0%)	0 (0%)	1 (5%)	
♦ ≥95th - 99th	2 (6.9%)	1 (6.7%)	2 (10%)	

10.9% of T1DM patients had decreased eGFR **(table 8)**, and there was no correlation between eGFR and blood pressure **(table 9)**.

Table (8) eGFR in the three T1DM groups

	eGFR (ml/min/1.73 m^2)		
	Normal	**Decreased**	**Increased**
Normoalbuminuric group n=29(%)	14 (48.3%)	5 (17.2%)	10 (34.5%)
Intermittent microalbuminuric group n=15(%)	9 (60%)	0 (0%)	6 (40%)
Persistent microalbuminuric group n=20(%)	14 (70%)	2 (10%)	4 (20%)

Table (9) Correlation between eGFR and blood pressure (SBP and DBP) centiles in T1DM patients

	eGFR (ml/min/1.73 m^2)	
	p value	**r value**
SBP (centiles)	0.798	**-0.033**
DBP (centiles)	0.308	**-0.129**

Table (10) Correlation between urine albumin/creatinine ratio and blood pressure centiles and eGFR in T1DM patients. Urine albumin/creatinine ratio did not significantly correlate with these variables.

	Urine albumin/creatinine ratio (mg/g creatinine)	
	p value	**r value**
SBP (centiles)	0.718	-0.046
DBP (centiles)	0.806	0.031
eGFR (ml/min/1.73 m^2)	0.874	-0.020

DISCUSSION

The most prevalent and severe microvascular consequence in individuals who have type 1 diabetes (T1DM) is diabetic kidney disease (DKD) **(Uwaezuoke and Ayuk, 2020).** DKD can be detected rather late by albuminuria. It has been stated that by the time albuminuria is identified, kidney damage has already occurred **(García-Carro et al., 2021)**. Remission of microalbuminuria to normoalbuminuria doesn't improve outcomes in type 1 diabetes (T1DM), which is linked to increasing risks of cardiovascular disease and lower eGFR **(de Boer et al., 2016)**. This led to the call for new circulating and urinary biomarkers in order to detect DKD earlier and improve prognosis **(García-Carro et al., 2021)**. We selected to test urine 8-Hydroxy-2-Deoxyguanosine (8-OHdG), and we preferred to study urine rather than serum 8-OHdG to be less invasive for the study participants.

The present study included ninety-one participants; 64 adolescents with T1DM were enrolled from the pediatric endocrine unit at the Cairo University Children's Hospital, and 27 healthy adolescents served as the control group. According to the results of their urine albumin/creatinine ratio (UACR), T1DM patients were classified into three groups: 29 normoalbuminuric patients, 15 intermittent microalbuminuric patients, and 20 persistent microalbuminuric patients. At the time of recruitment, the study participants' ages ranged from 10.2 to 19 years, and their mean ages were 14.84 ± 2.59 years, and more than 50% were females in the three groups.

The present study showed that urine 8-OHdG was significantly greater in T1DM patients in relation to the control group ($p<0.001$); in addition, urine 8-OHdG was significantly greater in persistent microalbuminuric patients than in intermittent microalbuminuric ($p<0.001$) and normoalbuminuric patients ($p=0.021$). Urine 8-OHdG and UACR were found to be strongly positively correlated ($p<0.001$ and $r=0.530$). Increased urine 8-OHdG excretion and its relationship to albuminuria in individuals at risk for DKD are indicators of a higher degree of oxidative DNA damage.

Results of the current study demonstrated that urine 8-OHdG can fairly distinguish between patients with normoalbuminuria and intermittent microalbuminuria; the best cutoff limit was 23 ng/ml; which had a 66.7% sensitivity and a 65.5% specificity. Also, urine 8-OHdG can fairly distinguish between patients with normoalbuminuria and persistent microalbuminuria; the best cutoff limit was 23.3 ng/ml; which had a 65% sensitivity and a 69% specificity. Urine 8-OHdG can't distinguish between patients with intermittent and persistent microalbuminuria, (area under the ROC curve=0.565=56.5%). This indicates that all adolescents with diabetes experiencing microalbuminuria whether intermittent or persistent develop oxidative damage to DNA of kidney cells, and are at risk of developing DKD.

Furthermore, 9 patients (31%) with normoalbuminuria in the current study have urine 8-OHdG levels above the cutoff limit (above 23 ng/ml). This data supports the suggestion that kidney cell DNA is damaged by oxidation prior to the development of microalbuminuria. Clinical and laboratory assessment of those 9 adolescents revealed diabetes

duration more than 10 years in 6 of them. Longitudinal follow up of those 9 patients is recommended to track progress to microalbuminuria and/or DKD.

The usual DKD presentations include long-standing diabetes, albuminuria without gross hematuria, reduction of estimated glomerular filtration rate (eGFR), and retinopathy. Decline of eGFR without albuminuria has been documented in type 1 and type 2 diabetes, in addition; it has been found to become more prevalent over time in the U.S. as diabetes prevalence rises **(ElSayed et al., 2023).**
In the current study, eGFR was decreased in 5 patients (17.2%) with normoalbuminuria; all of them had normal blood pressure (BP), and only 2 of them had high urine 8-OHdG level. Another 2 patients with persistent microalbuminuria had decreased eGFR, and both patients had normal BP and urine 8-OHdG level.

This study showed that 25% of T1DM patients were stunted (height SDS <-2), and stunting was more frequent in patients with persistent microalbuminuria (40%) than those with intermittent microalbuminuria (26.7%) or normoalbuminuria (13.8%). We suggest that decrease of linear growth can be considered an indicator of DKD risk.

There was no statistically significant correlation between urine 8-OHdG and mean HbA1c taken for the last 5 years before the study (p=0.056). This conclusion is in accordance with the findings of past studies **(Dandona et al., 1996)**. On the other hand, in patients with diabetes, **Goodarzi et al. (2010)** discovered a considerable correlation between urine 8-OHdG and HbA1c.

The present study showed no statistically significant correlation between eGFR and mean HbA1c over the previous five years (p = 0.265). **Lee et al. (2013)** observed that higher baseline HbA1c negatively affects eGFR, a dynamic effect, which varied among different CKD stages. However, when studied cross-sectionally; HbA1c was positively correlated with eGFR.

Absence of correlation between mean HbA1c in the last 5 years and eGFR in this study can be accounted for by the fact that mean HbA1c in the last 5 years was above the target (≥7%) in all patients with T1DM except 2 patients, which means that high HbA1c alone is not a risk factor for DKD. In addition, mean HbA1c during the first 2 years after diagnosis was not available in this study.

CONCLUSION

In comparison to the control group, T1DM patients had considerably higher mean urine 8-OHdG level. Urine 8-OHdG and urine albumin/creatinine ratio showed a strong positive correlation. The mean urine 8-OHdG was higher in persistent and intermittent microalbuminuric patients compared to those who had normoalbuminuria. Nine T1DM patients with normoalbuminuria had urine 8-OHdG level above the cutoff limit.

Recommendations

Follow up of the nine T1DM patients with normoalbuminuria and high urine 8-OHdG level is recommended to make sure that they will develop microalbuminuria later on, and at that time we can prove that urine 8-OHdG can be used for earlier detection of DKD. More studies are required to verify the current study's findings, in addition to simultaneously measure urine and serum 8-OHdG in order to evaluate the potential clinical applications of 8-OHdG as a biomarker for earlier detection of DKD.

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